

Appl. No. 10/071,349
Amdt. dated August 10, 2004
Reply to Office action of March 24, 2004

Amendments to the Specification:

Please replace paragraph [0166] with the following replacement paragraph:

[0166] The results from these experiments suggest, therefore, that the target (or targets) inhibited by the CD8⁺ suppressor molecule is (or are) one(s) which is (or are) active during ~~the latest~~ stages of the pseudotyped ~~viruses~~ virus life cycle subsequent to viral entry. ~~Possible~~ Primary targets ~~therefore including~~ include, but are not limited to, integration of viral DNA, transactivation from the proviral state, export of tat and/or rev into the cytoplasm and then back into the nucleus, and/or tat mediated enhancement of transcription

Please replace paragraph [0176] with the following replacement paragraph:

[0176] The present invention also provides a method for detecting a ~~CD8+~~ CD8⁺ suppressor molecule that has anti-HIV activity, the method comprising contacting a host cell with an env deficient HIV pseudotyped virus, comprising a reporter gene substituted for an HIV nef gene, such that said reporter gene is expressed in place of the HIV nef gene; contacting the host cell with a sample comprising (i) enriched ~~CD8+~~ CD8⁺ cells, (ii) a cell culture of ~~CD8+~~ CD8⁺ cells, or (iii) an extract or media component therefrom; and (c) measuring inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

Please replace paragraph [0181] with the following replacement paragraph:

[0181] The present invention also provides a diagnostic assay for monitoring clinical progression of HIV infection, said diagnostic assay comprising contacting a host cell with an env deficient HIV pseudotyped virus comprising a reporter gene substituted for an HIV nef gene such that said reporter gene is expressed in place of the HIV nef gene; contacting the host cell with samples from an HIV infection individual, said samples being collected successively from said individual; and measuring inhibition of reporter gene activity when each successive sample is contacted to the host cell, wherein a decrease in the ~~inhibition~~ inhibition of reporter gene activity when each successive sample is contacted to the host cell indicates progression of HIV infection.

Please delete paragraph [0184].

Please replace paragraph [0187] with the following replacement paragraph:

[0187] In another embodiment, the viral entry inhibitor is an antibody that disrupts the interaction between a CD4^+ CD4^+ cell surface receptor and a viral envelope protein. In a further embodiment, the antibody is a monoclonal antibody that specifically binds to the CD4^+ CD4^+ receptor. In another embodiment, the particular stage of replication is reverse transcription.

Please replace paragraph [0191] with the following replacement paragraph:

[0191] The present invention also provides a method for obtaining a preparation containing a CD8^+ CD8^+ suppressor molecule, said method comprising collecting conditioned media from cells expressing the CD8^+ CD8^+ suppressor molecule; fractionating media components of said conditioned media; and identifying a fraction having CD8^+ CD8^+ suppressor activity by a method comprising (i) contacting a host cell with a replication deficient pseudotyped HIV virus comprising a reporter gene operatively associated with an HIV promoter, (ii) contacting the host cell with a fractionated media component of said conditioned media, and (iii) measuring inhibition of reporter activity; wherein inhibition of reporter activity correlates with a fraction containing CD8^+ CD8^+ suppressor activity. In a further embodiment, the reporter gene is expressed during early proviral gene expression.

Please replace paragraph [0192] with the following replacement paragraph:

[0192] The present invention also provides a method for obtaining a preparation containing a CD8^+ CD8^+ suppressor molecule, said method comprising preparing an extract from a cell or cell line expressing the CD8^+ CD8^+ suppressor molecule; fractionating components of said extract; and identifying a fraction having CD8^+ CD8^+ suppressor activity by a method comprising (i) contacting a host cell with a replication deficient pseudotyped HIV virus comprising a reporter gene operatively associated with an HIV promoter, (ii) contacting the host cell with a fractionated component of said extract, and (iii) measuring inhibition of

reporter activity; wherein inhibition of reporter activity correlates with a fraction containing a ~~CD8+~~ CD8⁺ suppressor molecule. In a further embodiment, the reporter gene is expressed during early proviral gene expression.

Please replace paragraph [0194] with the following replacement paragraph:

[0194] In another embodiment, the pseudotyped virus is an env deficient pseudotyped virus. In a further embodiment, the pseudotyped virus is produced by a method which comprises co-transfecting DNA for said pseudotyped virus with a vector that encodes a viral envelope protein. In one embodiment, the viral envelope protein is an HIV Env protein. In another embodiment, the viral envelope protein is a non-HIV envelope protein. In another embodiment, the conditioned media or extract is ~~prepared~~ prepared from a lymphocyte cell clone that expresses a ~~CD8+~~ CD8⁺ suppressor molecule that inhibits HIV replication.

Please replace paragraph [0195] with the following replacement paragraph:

[0195] The present invention also provides a method for isolating a recombinant cDNA clone encoding a ~~CD8+~~ CD8⁺ suppressor molecule that inhibits HIV replication, said method comprising constructing a cDNA expression library using mRNA prepared from ~~CD8+~~ CD8⁺ T-lymphocytes that express a ~~CD8+~~ CD8⁺ suppressor molecule; and screening cDNA products from said cDNA expression library using a method comprising, for each of said cDNA products, (i) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter, (ii) contacting the host cell with a sample comprising a cDNA product from the cDNA expression library, and (iii) measuring inhibition of reporter gene activity; wherein inhibition of reporter gene activity indicates that a recombinant cDNA clone encodes a suppressor molecule that inhibits HIV.

Please replace paragraph [0197] with the following replacement paragraph:

[0197] In another embodiment, the method further comprises, before said screening step, a step of enriching the cDNA library by eliminating clones that hybridize to cDNAs prepared from mRNA of lymphocytes that do not express a ~~CD8+~~ CD8⁺ suppressor molecule.